

4.3 Network model for red blood cells

We have discussed that the cell is surrounded by a lipid bilayer which forms the cellular membrane. You have also seen that this lipid bilayer might be very soft. To structurally support the cell membrane, many cells have adopted an extremely elegant design concept by lining the intracellular side of the plasma membrane through an internal scaffold.

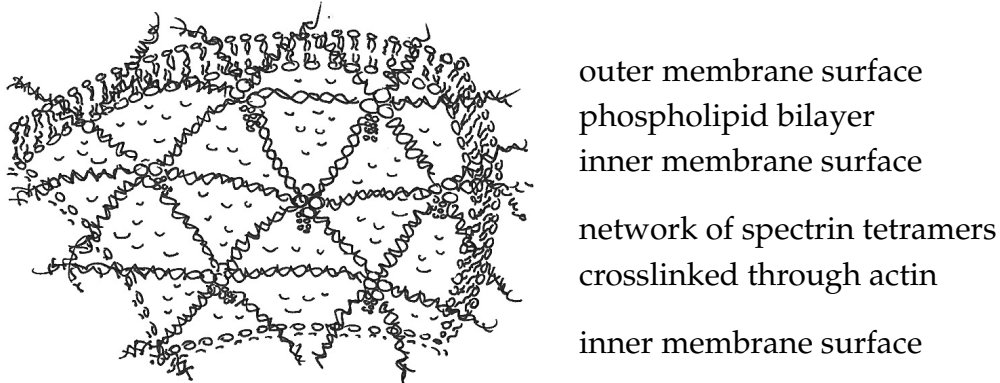


Figure 4.6: Microstructural architecture of the cell membrane of a red blood cell. A six-fold connected network of spectrin tetramers which are crosslinked through short actin filaments, anchored to the phospholipid bilayer, provides structural support to the inner cell membrane.

Erythrocytes, red blood cells, are maybe the most fascinating cells in the human body. They deliver oxygen to the body via the blood flow through the circulatory system. Erythrocytes take up oxygen in the lungs and release it while squeezing through the body's capillaries. Adult humans have about $2.5 \cdot 10^{13}$, 25 trillion, red blood cells; those form about a quarter of the total amount of cells in the human body. Adult human red blood cells are flexible biconcave disks without a nucleus. A typical human erythrocyte has a disk diameter of 6 - 8 μm , a thickness of 2 μm , a volume of 90 fL, and a surface of 136 μm^2 . It can swell to spherical shape of 150 fL, without membrane distension. The membrane of red blood cells plays a key role in regulating surface deformability, flexibility, and adhesion to other cells. These functions are highly regulated by its composition. The membrane of red blood cells is composed of three layers: (i) the exterior glycocalyx, which is rich in carbohydrates; (ii) the lipid bilayer with its transmembrane proteins; and (iii) the internal membrane skeleton, a structural network of spectrin tetramers composed of roughly 33,000 hexagons giving it an appearance of a microscopic geodesic dome.

Figure 4.6 illustrates a cell membrane which is internally supported by a six-fold connected network of polymeric chains. From a mechanical point of view, this internal scaffold which plays a crucial role in maintenance of plasma membrane integrity and cytoskeletal structure. A typical protein to form this supporting network structure is spectrin. At either end, spectrin tetramers are associated with short filaments of actin

which act as junctional complexes allowing the formation of the hexagonal mesh. The most prominent examples of cells with spectrin supported plasma membranes are erythrocytes, red blood cells. During their journey through the cardiovascular system, red blood cells have to squeeze through tiny thin capillaries which are much smaller in diameter than the cells themselves. How is that possible? Can their spectrin network help to explain their tremendous flexibility? Why are these networks so regular? Why are most biological networks six-fold or five-fold connected rather than four-fold connected? Can we explain these phenomena from a mechanical point of view?

Homogenization

In this section, we try to better understand the structural importance of spectrin networks. Our first goal is to express the overall macroscopic network properties of the spectrin lattice in terms of known microscopic material parameters, in this case, the stiffness of spectrin filaments. In theoretical and computational mechanics, this approach is extremely common; it is referred to as homogenization. The underlying theory is based on the Hill condition

$$W^{\text{mac}} \doteq W^{\text{mic}} \quad (4.3.1)$$

which states that upon deformation, the energy W^{mac} stored in the macroscopic continuum should be equivalent to the energy W^{mic} stored in the microstructural network. The macroscopic energy W^{mac} of a network-equivalent two-dimensional continuous sheet can be expressed in terms two contributions, one related to bulk extension, weighted by the bulk modulus κ , one related to shear, weighted by the shear modulus μ .

$$W^{\text{mac}} = \frac{1}{2} \kappa [\varepsilon_{xx} + \varepsilon_{yy}]^2 + \frac{1}{2} \mu [\varepsilon_{xx} - \varepsilon_{yy}]^2 + 2 \mu \varepsilon_{xy}^2 \quad (4.3.2)$$

In this equation, ε_{xx} and ε_{yy} are the normal strains in the x and y directions, respectively, and ε_{xy} are the shear strains, which we have introduced in the kinematics section 2.2.2. Now, the general idea is to compare this continuous macroscopic energy to the discrete microscopic network energy to express the overall bulk and shear moduli κ and μ in terms of the spectrin stiffness k . Spectrin polymers typically have an end-to-end length of $r = 75$ nm, a contour length of $L = 200$ nm, an approximate segment

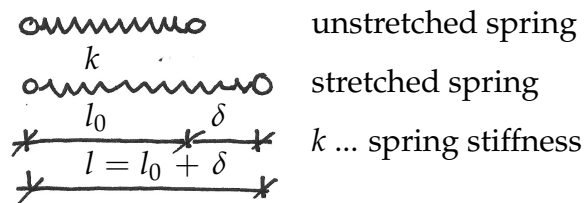


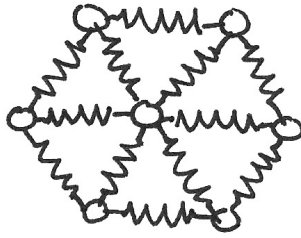
Figure 4.7: Spectrin can be modeled as Gaussian chain which we can conceptually replace by an equivalent linear entropic spring with a spring stiffness of $k = 3 k T N / L$. The strain energy of this spring can then be expressed as $W^{\text{spr}} = \frac{1}{2} k \delta^2$.

length of $l = 7.5\text{nm}$. These numbers tell us that $l \ll L$ and $r \ll L$, or, more specifically, that we can model spectrin as a Gaussian chain. We have seen that Gaussian chains are linear and that we can conceptually replace them by equivalent linear entropic springs with a spring stiffness of $k = 3kT N / L$, where k is the Boltzmann constant, T is the absolute temperature, N is the number of bonds, and L is the contour length. This really simplifies our model, because the network of entropic polymers can now simply be replaced by a network of linear springs. The free energy W^{spr} of a single spring then takes the following simple representation,

$$W^{\text{spr}} = \frac{1}{2} k \delta^2 = \frac{1}{2} k [l - l_0]^2 \quad \text{where} \quad \delta = l - l_0 \quad (4.3.3)$$

where δ denotes the change in filament length, i.e., the difference between the current length l and the original length l_0 , see figure 4.7. To evaluate equation , we need to characterize the microscopic network energy density W^{mic} , i.e., the energy per representative network area. This means we need to relate the energy of a single spring to the area for which it is representative. In the next two subsections, we demonstrate

six-fold connected network



four-fold connected network

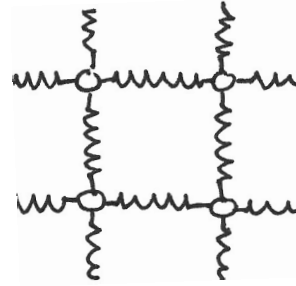


Figure 4.8: Microstructural architecture of a six-fold and four-fold connected network. The theory of homogenization helps to explain why nature prefers a six-fold connected network geometry.

this procedure for six-fold and for four-fold connected network as illustrated in figure 4.8, and characterize both network geometries in terms of their homogenized material properties. Because it makes life much easier, we will look at network extension and network shear individually. The conceptual separation of extension and shear is, by the way, a very common approach in material science, theoretical, and applied mechanics.

4.3.1 Six-fold connected network

Extension. Let's first consider the case of extension, and relate the first term of the macroscopic free energy in equation (4.3.2) to the corresponding microscopic free energy term. To do so, we look at the smallest representative unit of the hexagonal network, the so-called representative volume element or RVE, which consists of a single triangle. If we extend this representative triangle isotropically, see figure 4.9, left, the required network energy density

$$W^{\text{mic}} = \frac{\sum_{i=1}^3 W_i^{\text{spr}}}{\sum_{i=1}^3 A_i^{\text{spr}}} \quad (4.3.4)$$

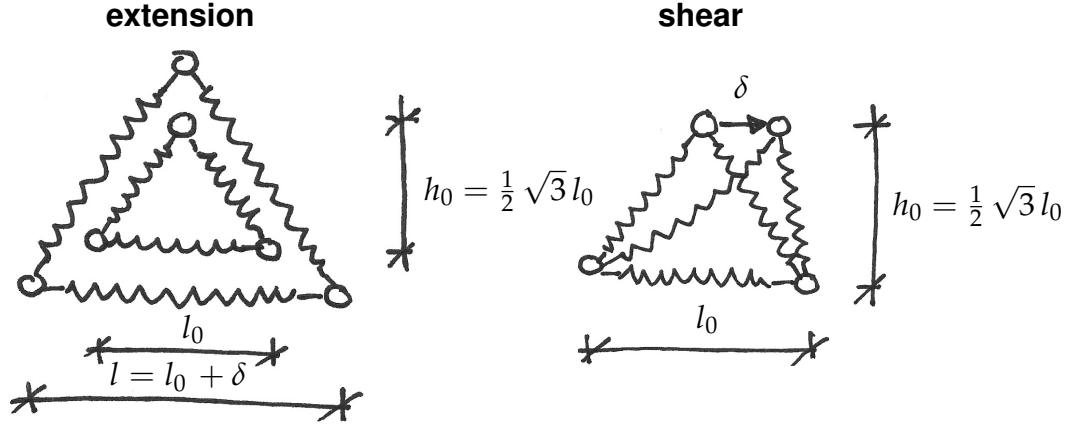


Figure 4.9: Kinematics of extension and shear for six-fold connected network.

is equal to the energy required to stretch all three individual chains

$$\sum_{i=1}^3 W_i^{\text{spr}} = 3 W^{\text{spr}} = 3 \left[\frac{1}{2} k \delta^2 \right] \quad (4.3.5)$$

divided by the area $3 A^{\text{spr}}$ covered by these three chains which is illustrated in figure 4.10, left.

$$\sum_{i=1}^3 A_i^{\text{spr}} = 3 A^{\text{spr}} = 2 A^{\text{triangle}} = 2 \left[\frac{1}{2} l_0 h_0 \right] = 2 \left[\frac{1}{2} l_0 \left[\frac{1}{2} \sqrt{3} l_0 \right] \right] = \frac{1}{2} \sqrt{3} l_0^2 \quad (4.3.6)$$

This means, that the microscopic free energy density (4.3.4) takes the following form.

$$W^{\text{mic}} = \frac{3 \frac{1}{2} k \delta^2}{\frac{1}{2} \sqrt{3} l_0^2} = \sqrt{3} k \left[\frac{\delta}{l_0} \right]^2 \quad (4.3.7)$$

For the six-fold connected network, the micro-to-macro relation for the extension term then follows from equation (4.3) by using the micro-to-macro kinematics

$$\varepsilon_{xx} = \delta / l_0 \quad \varepsilon_{yy} = \delta / l_0 \quad \varepsilon_{xy} = 0 \quad (4.3.8)$$

such that

$$W^{\text{mac}} \doteq W^{\text{mic}} \quad \text{thus} \quad \frac{1}{2} \kappa \left[\frac{\delta}{l_0} + \frac{\delta}{l_0} \right]^2 = \sqrt{3} k \left[\frac{\delta}{l_0} \right]^2 \quad \rightarrow \quad \underline{\underline{\kappa = \frac{1}{2} \sqrt{3} k}}. \quad (4.3.9)$$

This relation tells us that the macroscopic bulk modulus κ , i.e., the overall network resistance to extension, scales linearly with the microscopic spring stiffness k , and that the corresponding scaling factor is $\sqrt{3} / 2$.

Shear. Now, we can repeat this homogenization procedure for the shear term. Unfortunately, this is a bit more tricky. Again, we start by using the definition of the microscopic free energy (4.3.4). To characterize the stretches of the three individual springs, we can again consider a representative triangle, however, this time, to induce shear,

we just shear its upper node, see figure 4.9, right. At the same time, both lower nodes remain fixed. Given this deformation, how much do the individual springs contribute? Figure 4.10, right, illustrates the new spring length $l = \sqrt{[h_0]^2 + [l_0/2 + \delta]^2} = l_0 + \delta/2$ which can then be inserted in the spring energy calculation.

$$\sum_{i=1}^3 W_i^{\text{spr}} = \underbrace{\frac{1}{2} k \left[+\frac{1}{2} \delta \right]^2}_{\text{left spring}} + \underbrace{\frac{1}{2} k \left[-\frac{1}{2} \delta \right]^2}_{\text{right spring}} + \underbrace{0}_{\text{lower spring}} = \frac{1}{4} k \delta^2 \quad (4.3.10)$$

Using the same representative area as in equation (4.3.6)

$$\sum_{i=1}^3 A_i^{\text{spr}} = 3 A^{\text{spr}} = 2 A^{\text{triangle}} = 2 \left[\frac{1}{2} l_0 h_0 \right] = 2 \left[\frac{1}{2} l_0 \left[\frac{1}{2} \sqrt{3} l_0 \right] \right] = \frac{1}{2} \sqrt{3} l_0^2 \quad (4.3.11)$$

we obtain the following shear term for the microscopic network energy density.

$$W^{\text{mic}} = \frac{\frac{1}{4} k \delta^2}{\frac{1}{2} \sqrt{3} l_0^2} = \frac{\sqrt{3}}{6} k \left[\frac{\delta}{l_0} \right]^2 \quad (4.3.12)$$

For the six-fold connected network, the micro-to-macro relation for the shear term follows from equation (4.3) by using the micro-to-macro kinematics

$$\varepsilon_{xx} = 0 \quad \varepsilon_{yy} = 0 \quad \varepsilon_{xy} = \frac{1}{2} [u_{x,y} + u_{y,x}] = \frac{1}{2} \left[\frac{\delta}{\frac{1}{2} \sqrt{3} l_0} + 0 \right] = \frac{1}{\sqrt{3}} \frac{\delta}{l_0} \quad (4.3.13)$$

such that

$$W^{\text{mac}} \doteq W^{\text{mic}} \quad \text{thus} \quad 2\mu \left[\frac{1}{\sqrt{3}} \frac{\delta}{l_0} \right]^2 = \frac{\sqrt{3}}{6} k \left[\frac{\delta}{l_0} \right]^2 \quad \rightarrow \quad \underline{\underline{\mu = \frac{1}{4} \sqrt{3} k}}. \quad (4.3.14)$$

This implies that the macroscopic shear modulus μ , i.e., the resistance to shear, scales linearly with the microscopic spring stiffness k whereby the corresponding scaling factor is $\frac{1}{4} \sqrt{3}$. To gain a better feeling for these values, we will now look at a four-fold connected network.

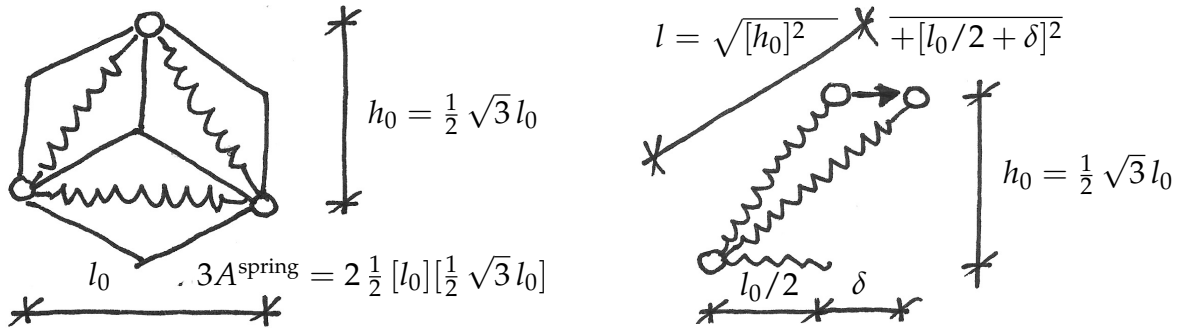


Figure 4.10: Illustration of representative area of three chains $3 A^{\text{spring}} = \sqrt{3}/2 l_0^2$ in six-fold connected network model, left. Illustration of deformed spring length $l = l_0 + \delta/2$ in six-fold connected network model subjected to shear, right.

4.3.2 Four-fold connected network

Extension. We follow the same approach as before and investigate the smallest representative unit of the network, the representative volume element or RVE, which consists of a single square as illustrated in figure 4.11, left. If we extend this square network isotropically, the required network energy density

$$W^{\text{mic}} = \frac{\sum_{i=1}^4 W_i^{\text{spr}}}{\sum_{i=1}^4 A_i^{\text{spr}}} \quad (4.3.15)$$

is equal to the energy required to stretch all four individual chains

$$\sum_{i=1}^4 W_i^{\text{spr}} = 4 W^{\text{spr}} = 4 \left[\frac{1}{2} k \delta^2 \right] = 2 k \delta^2 \quad (4.3.16)$$

divided by the representative square area $4 A^{\text{spr}}$.

$$\sum_{i=1}^4 A_i^{\text{spr}} = 4 A^{\text{spr}} = 2 A^{\text{square}} = 2 l_0^2 \quad (4.3.17)$$

With the help of these considerations, we can reformulate the microscopic free energy density (4.3.15) in the following form.

$$W^{\text{mic}} = \frac{2 k \delta^2}{2 l_0^2} = k \left[\frac{\delta}{l_0} \right]^2 \quad (4.3.18)$$

The micro-to-macro relation for the extension term of the four-fold connected network again follows from equation (4.3) by using the micro-to-macro kinematics

$$\varepsilon_{xx} = \delta / l_0 \quad \varepsilon_{yy} = \delta / l_0 \quad \varepsilon_{xy} = 0 \quad (4.3.19)$$

such that

$$W^{\text{mac}} \doteq W^{\text{mic}} \quad \text{thus} \quad \frac{1}{2} \kappa \left[\frac{\delta}{l_0} + \frac{\delta}{l_0} \right]^2 = k \left[\frac{\delta}{l_0} \right]^2 \quad \rightarrow \quad \underline{\underline{\kappa = \frac{1}{2} k}} \quad (4.3.20)$$

For the four-fold connected network, the macroscopic bulk modulus κ turns out to be half the microscopic spring stiffness k .

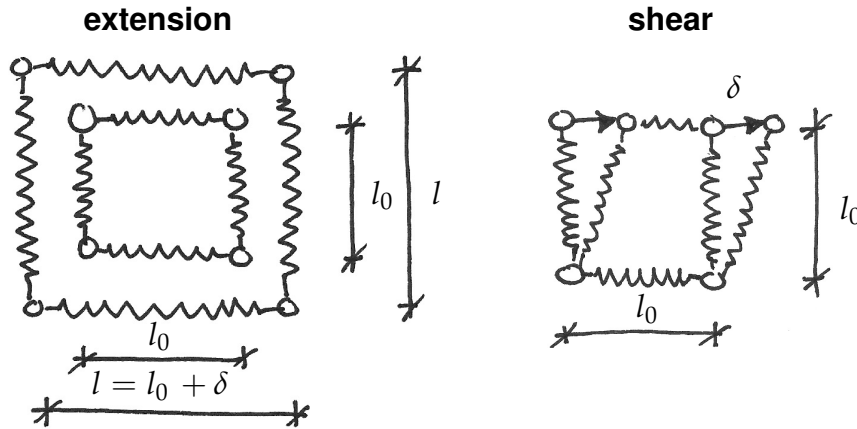


Figure 4.11: Kinematics of extension and shear for four-fold connected network.

Shear. Now, let's look at the shear term using the definition of the microscopic free energy (4.3.15). To characterize the stretches in the four individual springs, we consider the representative square as RVE, on which we induce shear by moving its upper two nodes while both lower nodes remain fixed as illustrated in figure 4.11, right. How much does the energy stored in the individual springs contribute to the stored energy of the network?

$$\sum_{i=1}^4 W_i^{\text{spr}} = \underbrace{2 \cdot \frac{1}{2} k \delta^2}_{\text{vertical springs}} + \underbrace{2 \cdot 0}_{\text{horizontal springs}} = k \delta^2 = 0 \quad (4.3.21)$$

Surprisingly, this energy term seems to be zero! Why does the shear contribution to the microscopic network energy vanish? In the small strain limit, the new spring length l is equal to the original length l_0

$$l = \sqrt{l_0^2 + \delta^2} \approx l_0 \quad \text{such that} \quad \delta = 0. \quad (4.3.22)$$

This implies that

$$W^{\text{mac}} \doteq W^{\text{mic}} \quad \text{where} \quad W^{\text{mic}} = 0 \quad \rightarrow \quad \underline{\underline{\mu = 0}} \quad (4.3.23)$$

i.e., the **four-fold connected network cannot store energy upon shearing**. Regardless of the spring stiffness k , when subjected to shear, the microstructural network just collapses. You can easily try this by building a four-fold connected network yourself and trying to shear it. There is virtually no resistance! Unlike the six-fold connected network, the four-fold connected network does not recover its original shape when subjected to shear. It is incapable to store mechanical energy!

What does this tell us about red blood cells? Why is their membrane supported by a six-fold connected network? A six-fold connected network can easily store and release energy. This mechanical feature is absolutely essential for red blood cells as they are being squeezed and released from small capillaries. By structurally supporting the cell membrane through a six-fold connected network, nature has found an extremely elegant and energetically favorable way to equip these cells with an outer layer that can be extremely resistant to tension, while at the same time being extremely flexible in shear.